HBV-TAG

2021 CONFERENCE

Hepatitis B Virus: Clinical Implications of Genotypes and Viral Mutations

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Conflicts of Interest

- I have no conflicts of interest pertaining to this presentation

Outline of this Presentations

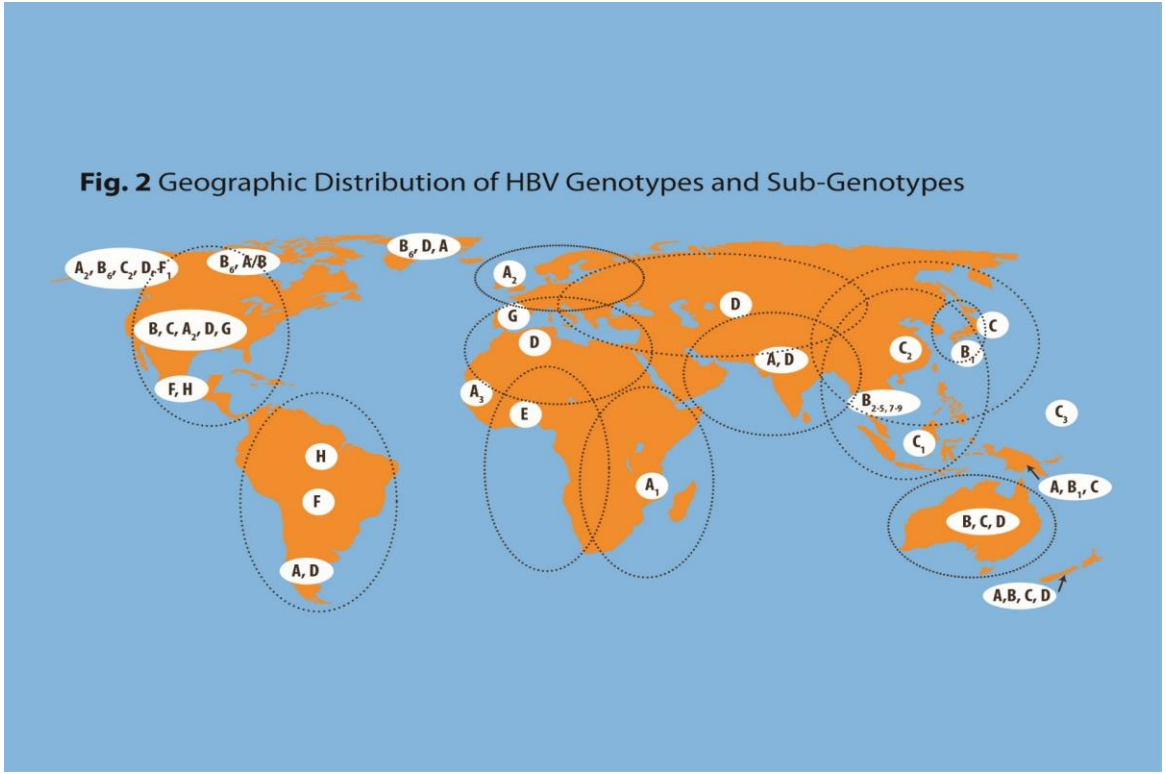
- Hepatitis B Virus Genotypes: What are the definitions, what are their global distributions and what are the clinical implications?
  - Are certain HBV genotypes associated with increase risk of developing cirrhosis or hepatocellular carcinoma (HCC)?
  - Are HBV genotypes associated with response to antiviral therapy?

- Hepatitis B mutations:
  - Precore mutations. Do they influence disease outcomes?
  - Core promoter mutations. Do they predict worsening liver inflammation and risk of HCC
  - HBsAg vaccine escape mutations: Do these mutants have us “Shaking in our Boots”, dumping all our current vaccines and devising new escape mutant proof vaccines?

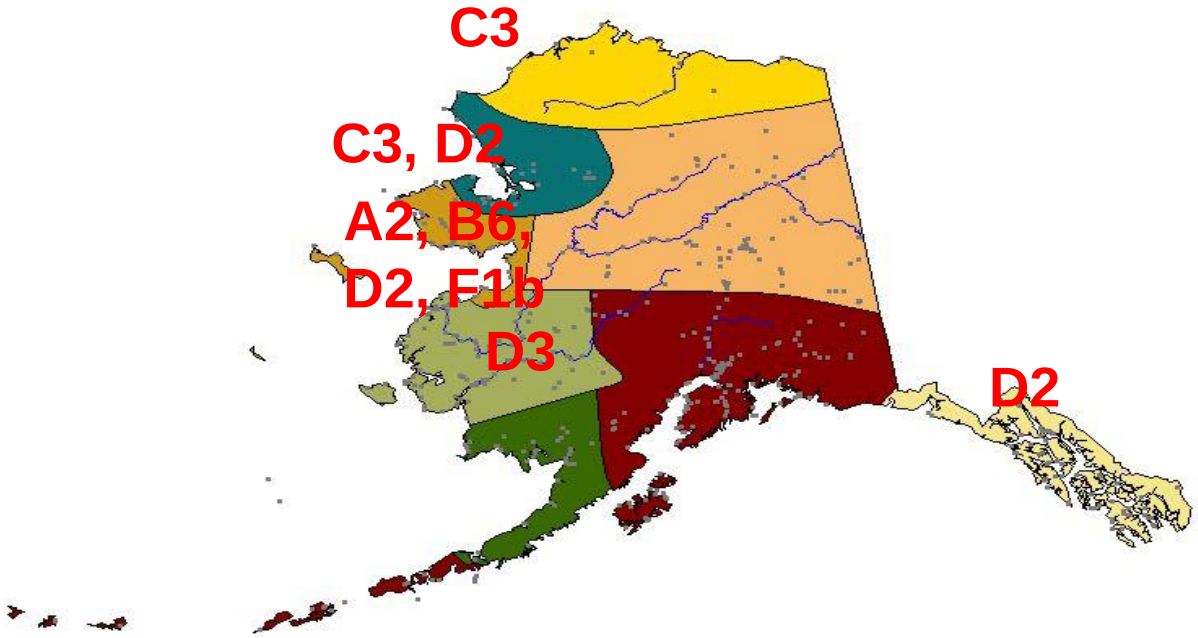
HBV Genotypes: What is the Definition?

- There are 8 HBV genotypes (A through H) which differ by >8% from each other in their genetic DNA base-pair sequences
  - For each genotype, there may be one of more sub-genotypes which vary by between 4% and 8% in their DNA base pair sequences
    - Within the sub-genotypes, some mutations appear to have a significant correlation with disease outcome, while others appear do not have any or very little influence
    - Because there are many confounding risk factors including age, sex, exposure to external toxins such as aflatoxin, family history, degree of underlying liver fibrosis, HBV Viral level and others, it is often difficult to attribute the degree of risk that individual genotypes convey.
- HBV genotypes also correspond to response to interferon therapy but not significantly to oral nucleoside(tide) inhibitors

Global Distribution of HBV Genotypes



Geographic Distribution of HBV Genotypes in Alaska Natives



Genotype B

- Genotype B comes in 2 forms
  - B1 and B6: The pure B genotype
    - B1 is found in Japan
    - B6 is found in Alaska and other Arctic Regions. Genetic distance using molecular clock studies suggest that B6 likely came from Japan and is about a millennium in distance
  - B2-B5: Contains a piece of genotype C recombined into the core region of the virus

Clinical Significance of HBV B Genotypes

- B1: Studies in Japan have found the incidence of HCC is significantly lower for those infected with B1 in Japan compared to those infected with genotype C and age of HCC occurs a decade later
- B6 Alaska: No cases of HCC have yet been identified but the number of infected persons is low

HBV Genotypes and Risk of Developing Cirrhosis

- In multiple case-control studies HBV genotype C is associated with an increase risk of developing cirrhosis and HCC than genotype B and other genotypes. Possible reasons:
  - Seroclearance of HBeAg occurs at significantly older age (mean 45) for those infected with HBV genotype C than with genotypes A, B, D and F allowing more years for higher HBV DNA levels to contribute to amount of HBV integration in host hepatocytes plus inflammation leading to cirrhosis. (Livingston et al. Hepatology)
  - Both case control and prospective studies have shown a higher risk of cirrhosis in persons infected with genotype C than other HBV genotypes

HBV genotypes and Risk of HCC: Population Prospective and Case-Control Studies

- Lower risk of HCC: Genotypes B, Japan (B1), Alaska B6 and D Arctic but D subtypes found all over the world
- Intermediate risk of HCC: A2 found in US, Arctic, Europe
- Higher risk of HCC:
  - A1, A3 and E found in Africa (however aflatoxin exposure may be a strong co-factor here)
  - B Asia (B2-5) is a recombination of sequences from genotype C into the core region of genotype B
- Highest risk of HCC: C (all subtypes?) and F1b
  - Role of underlying liver disease: HCC in persons with genotype C is primarily also associated the presence of cirrhosis
  - HCC in F1b can occur in the presence of stage 1 fibrosis alone.

Demographics, outcomes, and person-years of follow-up by genotype (Alaska, 1983-2012)

Characteristics	Genotype					
	A	B	C	D	F	Overall
HBV	**154**	**45**	**74**	**650**	**217**	**1142**
Median age entry†	24.5	52.5	24.2	21.2	17.6	25.9
HCC	**5**	**0**	**10**	**6**	**22**	**43**
Median age HCC	59.8	--	59.2	54.2	23.0	44.7
Deaths	**44**	**26**	**27**	**185**	**64**	**346**
Median age death	53.5	77.4	61.9	56.4	39.9	56.4
Total pyrs at risk^	**3884**	**917**	**1813**	**15933**	**5183**	**27729**
Median	29.1	20.6	27.0	29.1	29.8	29.1

†Entry into study is 3/1/1983 or date of first HBsAg positive test.
^Person years at risk is period from entry into study to HCC diagnosis, death, or end of study period on 12/31/2012.

Ching, Gounder et al. Liver International 2016

Genotype F disproportionately affects young. Other Genotypes affecting older persons.

Adjusted odds ratios, HCC (Alaska, 1983-2012)

	Incidence**	HCC aOR††	HCC 95% CI
Age Group (years)*		*p=0.043*	
<40	1.05	1.0	--
40-60	1.43	2.25	(0.99, 5.12)
≥60	3.75	2.81	(0.99, 7.96)
Sex		*p=0.089*	
Female	0.99	1.0	--
Male	1.81	1.83	(0.91, 3.65)
HBV Genotype		*p<0.001*	
B/D	0.38	1.0	--
A	1.29	4.05	(1.21, 13.6)
C	5.52	18.1	(6.29, 52.1)
F	4.24	14.6	(5.74, 36.9)

†C.I. Confidence Interval. ††aOR = adjusted odds ratio, controlling for age, sex, and HBV genotype.
*HCC age represents age at entry into study. **HCC incidence is per 1000 person-years at risk.

An Association Between Core Mutations in Hepatitis B Virus Genotype F1b and Hepatocellular Carcinoma in Alaskan Native People

HEPATOLOGY, VOL. 69, NO. 1, 2019

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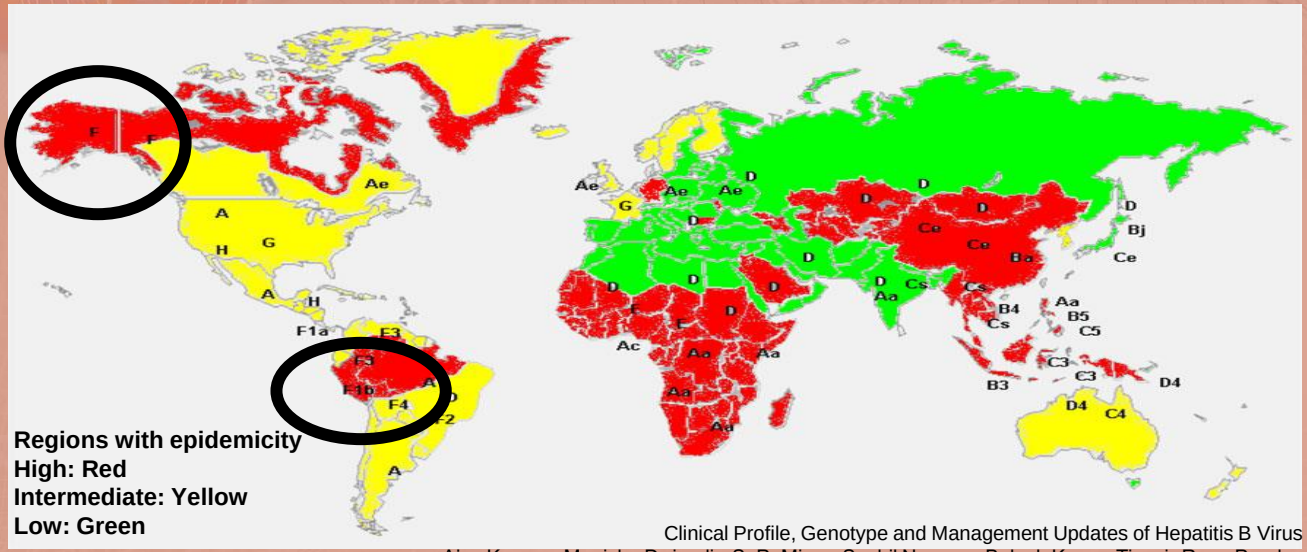


HBV Genotype F1b and HCC

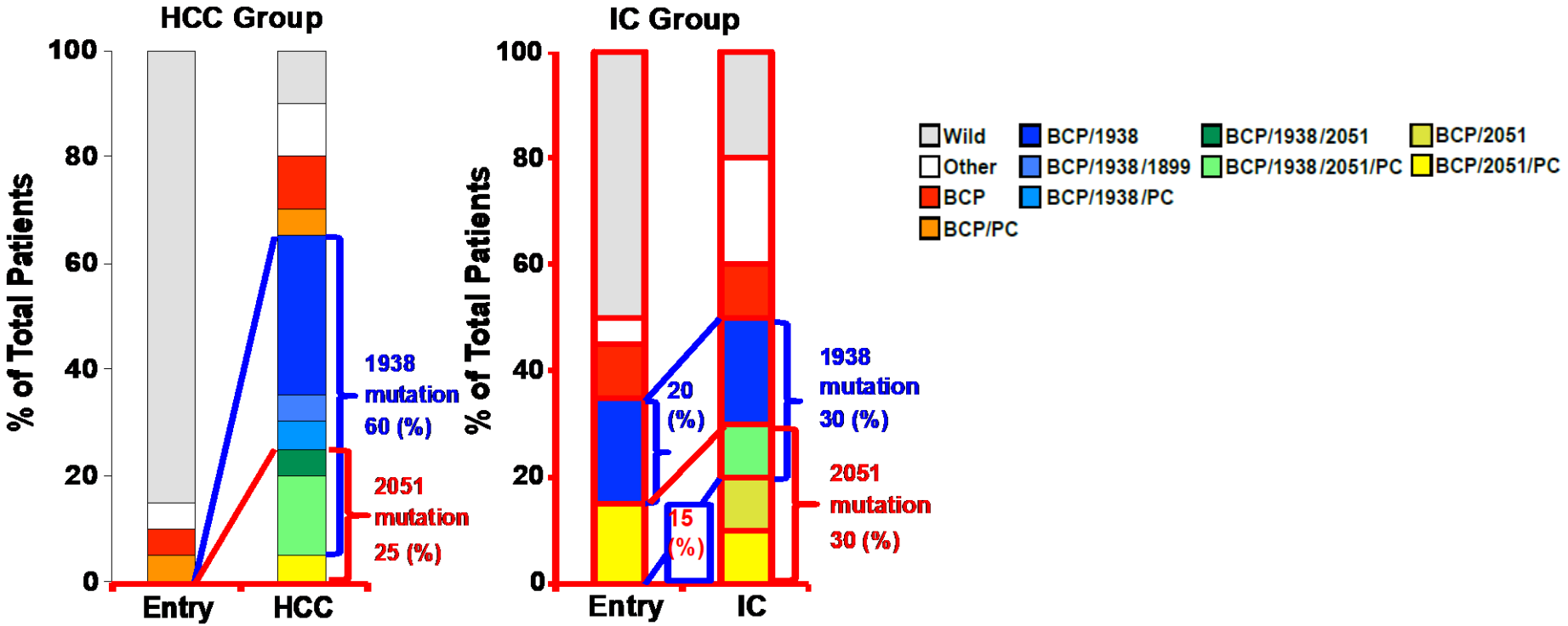
Dr. Tanaka's group from the City Medical College of Nagoya Japan compared clinical and virological characteristics between 20 HCC patients and 20 inactive carriers (IC) in serial samples collected before and after HCC development infected with genotype F1b by producing replicons and introducing them into human hepatocytes in tissue culture and then into mice with humanized livers

Hayashi S et al. Hepatology 2019;69:19-33

Emergence of 2051 Core Mutation

- In the earliest samples both of those who developed HCC and controls infected with genotype F1b, no 2051 mutations were evident
- 2051 mutations emerged and replicons with 2051 were associated with higher rates of HBV replication and hepatocyte damage in human tissue culture cells

Core 1938 and 2051 mutations mostly occurred in combination with BCP and PC mutations.

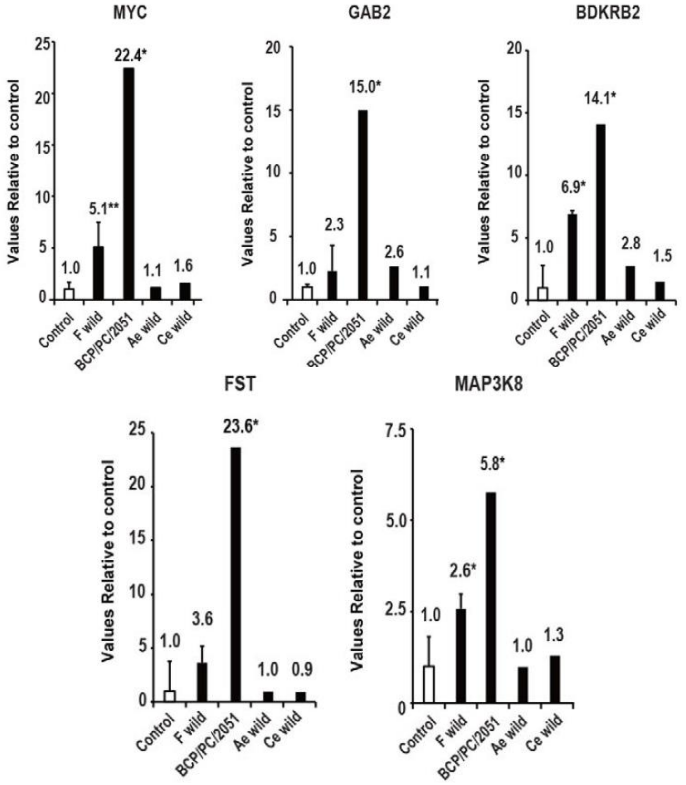


Introduction of HBV Core Mutation into Mice with Humanized Livers

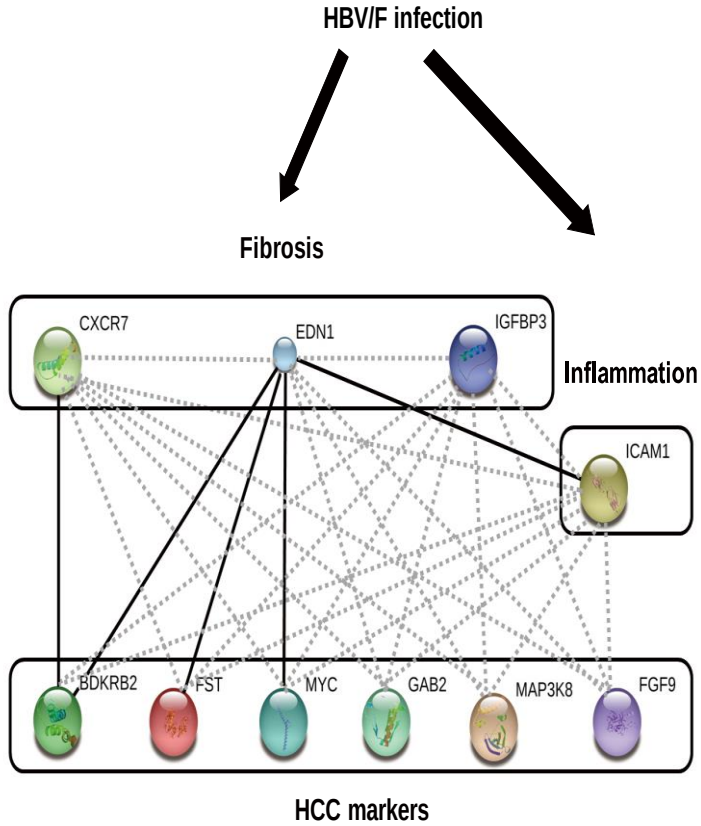
- When livers replicons containing he 2051 mutations were introduced into mice with humanized liver, over the next year, multiple oncogenic genes emerged. These mice did not liver long enough to determine if HCC would occur.

The BCP/PC/2051 mutant up-regulates HCC progression via the fibrosis and inflammation

Up-regulated gene expression in human hepatocytes



*p<0.01, and **p<0.05 in comparison with Control.



HBV Precore Mutations

- More common in those infected with HBV genotype A
- It is controversial whether or not these mutations contribute to worsening liver inflammation and fibrosis
  - In Europe, these mutations have been found to increase risk of liver fibrosis in case-control studies
  - In Taiwan, in the REVEAL study, a population-based prospective cohort study they did not significantly increase cirrhosis risk

HBV Core Promoter Mutations

- Multiple case-control and the population-based REVEAL study found that the presence of mutations in the core-promoter area were significantly associated with a higher risk of cirrhosis and HCC independent of age, genotype and HBV DNA levels

Vaccine Escape Mutations in the HBV Surface Antigen Gene

- Vaccine escape HBV mutations have been identified that blunt response to current recombinant vaccines
- These mutations in the past have appeared primarily in infants who received HBV vaccine plus HBIG at birth with high viral levels.
  - Though we have no data on infants whose mothers also received Tenofovir prior to delivery but would expect these mutations to be far less likely in these instances
- Levels of HBV DNA in those infants who develop HBsAg+ vaccine escape mutations are very low, < 1,000 IU/ml making person to person transmission highly unlikely
- In the 1980's few prominent experts got up at international liver meetings and advocated stopping all hepatitis B vaccinations to prevent an epidemic of mutants which could result in Godzilla like complications for the world until they could develop and test new vaccines effective against these. Fortunately. no one paid attention to these suggestions.

Surveillance for HCC in HBV Infected Persons: Does One Size Fit All?

- Current HCC surveillance recommendations published by International Liver Disease Societies recommend screening for HCC with ultrasound or other imaging plus Alpha-fetoprotein (AFP) {APASL} or imaging with optional AFP (AASLD and EASL) every 6 months in:
  - Patients of Asian descent starting age 40 males, 50-years females
  - Patients of African descent males age 20, no formal recommendation for females.
  - No formal recommendations for other racial or ethnic groups except for those with cirrhosis based on a paucity of data
- More studies are needed to craft specific screening that incorporate other known and yet unproven risk factors such as HBV genotype, viral mutations and other racial and ethnic origins.

Conclusions

- Specific viral characteristics including HBV genotype, pre-core and core promoter mutations appear to have significant associations with the risk of developing cirrhosis and HCC in persons with chronic HBV infection.
- More studies, especially prospective long-term outcome, but also case-control, are needed to develop better tools to follow HBV infected persons in order to intervene more timely with antiviral therapy and individualize HCC surveillance intervals.
- Support of population-based prospective studies conducted in multiple global regions are needed that also include cost-effectiveness analysis to better care for HBV infected persons
- Unfortunately, no exciting TV series or movies featuring HBV vaccine escape mutants were ever produced in the 1980s